they block the recycling step. Wells and coworkers also found that Amot can inhibit the GAP activity of Rich1, and a partial depletion of Amot stabilizes the junctions—possibly because the increased rate of Cdc42 GTP hydrolysis leads to more efficient recycling. Thus, the sorting decision might be regulated by the level of Amot that is associated with Rich1. However, as Amot, and possibly Rich1, do not arrive at the tight junctions until several hours after their initial assembly, the sorting and recycling process would not begin until the junctions were intact. Thus, establishment would be separated from maintenance.

The link to Cdc42 is particularly intriguing. Although this small GTPase was, like Rac and Rho, initially implicated in remodeling of the actin cytoskeleton, it is localized primarily to the Golgi apparatus and seems to influence vesicle traffic in numerous ways (Rodriguez-Boulan et al., 2005). It binds coatomer and regulates the interaction of COP1 with dynein (Chen et al., 2005). In addition, dominant-interfering mutants of Cdc42 inhibit basolateral vesicle sorting and stimulate apical delivery. So it is not such a stretch to imagine that it also regulates the decision as to the destination of endocytosed tight junction proteins.

Of course, many questions remain to be addressed. Clearly, the vesicles involved in endocytosis and recycling of the tight junction proteins must be distinct from those that recycle E-cadherin because manipulation of Rich1 or Amot does not interfere with adherens junction assembly or maintenance. But do these vesicles have a specialized coat? Are specific adapters involved? Do the vesicles bud from the tight junction itself, or from above or below it? Do Patj and Pals1 recruit Rich1/Amot to the vesicle exit site, or do they behave as cargo that recycles in association with Crumbs? Are Rich1 and Amot part of a signaling pathway that regulates the stability of tight junctions during epithelial-mesenchymal transitions? Is a Cdc42 switch involved in other specialized vesicle sorting decisions, for instance at the bud neck in *S. cerevisiae*? The paper by Wells and colleagues hints at an entirely new level of control for vesicle sorting that will provide a rich vein for future studies.

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Descrambling DSCAM Diversity

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Neuronal processes exhibit exquisitely complex branching patterns crucial for the formation of distinct neural circuits. In this issue of *Cell*, Chen et al. (2006) show that the isoform diversity of the Dscam protein in *Drosophila* is required to establish stereotypical axonal branching patterns, suggesting that nonrandom expression of *Dscam* alternative splice variants determines neural connectivity.

The human brain possesses around a trillion neurons that together form approximately 10^{15} synaptic connections. To achieve this extraordinary degree of connectivity, neurons must assume complex morphologies to establish specific synaptic contacts in distinct patterns that define functional circuits. The generation of precise neural connectivity patterns depends critically upon the repertoire of guidance cues

and their cell-surface receptors. In principle, a limited number of guidance cues operating in a combinatorial fashion could facilitate proper pathfinding and targeting of a very large number of neurons. However, certain neuronal proteins exhibit a remarkable degree of isoform diversity, including Dscam in *Drosophila* and cadherin-related proteins and neurexins in vertebrates. The need for considerable isoform complexity of these proteins in invertebrates can be appreciated in the context of the complex but highly stereotyped patterns of branching observed in *Drosophila* mechanosensory, motor, and olfactory neuronal processes. A similar degree of conservation in the branching pattern of vertebrate neuronal processes has yet to be demonstrated. However, diverse vertebrate neuronal subtypes can be effectively classified based on axonal or dendritic morphology, and conserved local cortical synaptic circuits have been observed (Kozloski et al., 2001). These results suggest that vertebrates may also require the ability to reproducibly generate complex branching patterns during neural development. It is therefore highly significant that Chen et al. (2006) show, in this issue of *Cell*, that molecular diversity of Dscam isoforms is critical for complex axonal targeting.

The fly *Dscam* gene includes 95 variable exons that have the potential to produce close to 40,000 distinct protein isoforms through alternative splicing. Dscam is a member of the immunoglobulin (Ig) superfamily and contains ten Ig and six fibronectin domains and single transmembrane and cytoplasmic domains (Schmucker et al., 2000). *Dscam* includes variable regions located in Ig domains 2, 3, and 7, each encoded by multiple exons. Phenotypic analyses exploiting various neural systems in *Dscam* mutant flies implicate Dscam in diverse processes including the regulation of axonal and dendritic branching and axonal targeting and fasciculation (the bundling of axons into tracts; Hummel et al., 2003; Schmucker et al., 2000; Wang et al., 2002; Zhu et al., 2006). In the *Drosophila* mushroom body—a higher brain center that processes olfactory information—axons initially project as part of a single fascicle in the central peduncle region and then bifurcate, sending branches to the medial and dorsal mushroom body lobes. In the absence of Dscam protein, mushroom body neurons often fail to segregate their axon branches into the dorsal and medial mushroom body lobes, and taken together with additional experiments, it seems likely that Dscam mediates repulsive interactions between newly formed mushroom body axon branches (Wang et al., 2002; Zhan et al., 2004). Remarkably, Dscam isoforms are capable of homophilic interactions showing exquisite binding specificity, to the point that isoforms differing only by a few amino acids exhibit no, or at best very weak, protein-protein associations (Wojtowicz et al., 2004). Examination of Dscam isoform expression profiles in single mushroom body neurons reveals that individual mushroom body neu-

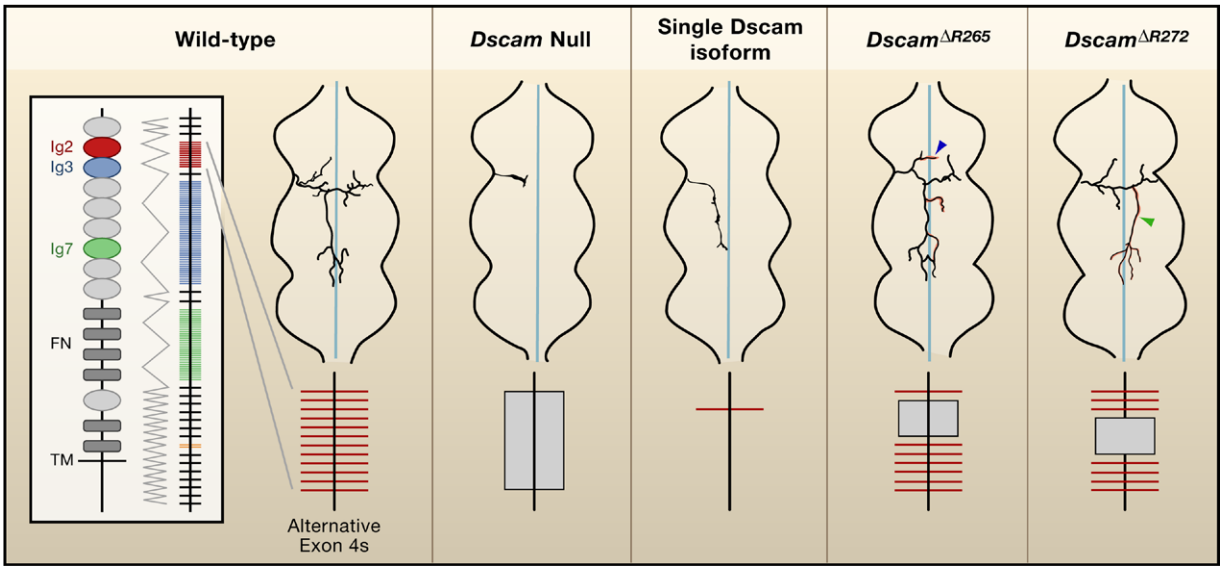

Figure 1. Dscam Diversity Is Required for Mechanosensory Axon Patterning

A schematic depiction of post-Scutellar (pSc) mechanosensory neuron projections into the adult fly thoracic CNS. The first panel shows the wild-type trajectory of a pSc mechanosensory neuron and also the molecular organization of the *Dscam* gene and protein. The *Dscam* gene has four sets of alternative exons (each set represented by a different color). Only one exon from each set gets spliced into an mRNA. The Dscam protein has ten Ig-like domains, six fibronectin type III domains (FN), and a transmembrane domain (TM). The remaining panels show pSc mechanosensory neurons in *Dscam* mutant flies with exon 4 isoform diversity indicated by an expanded view of the exons present in each mutant. The light gray boxes indicate deletions. Ectopic or misrouted branches are highlighted in red, branches prevalent in either *Dscam* deletion mutant (but not both) are highlighted by blue and green arrowheads, and the blue line denotes the CNS midline.

rons express multiple Dscam isoforms and that the subset of Dscam isoforms expressed by different mushroom body neurons is largely nonoverlapping (Neves et al., 2004; Zhan et al., 2004). Thus, Dscam isoforms could provide a molecular tag capable of allowing axon branches to make self/nonself assessments, allowing for repulsive or adhesive interactions dependent upon the strength of the homophilic or heterophilic associations mediated by Dscam. Is Dscam isoform diversity indeed required for bifurcation of mushroom body axons? Analysis of *Dscam* mutants that reduce Dscam isoform complexity 4-fold revealed no discernable mushroom body axon pathfinding defects (Wang et al., 2004). Further, expression of a single Dscam isoform in individual *Dscam*-deficient mushroom body neurons partially rescues branching defects (Wang et al., 2004; Zhan et al., 2004), although expression of a single isoform in many mushroom body neurons gives rise to a severe dominant phenotype (Zhan et al., 2004). Hence, although these studies suggest that mushroom body neurons must express isoforms different from their neighbors for proper axon branch segregation, there does not appear to be a requirement for any one isoform, or specific subset of them, in a given mushroom body neuron. There are, however, inherent differences in binary mushroom body axon branching compared to more complex guidance decisions made by other neurons, where Dscam isoform diversity may be required to navigate choice points.

Chen et al. (2006) address this crucial issue of Dscam isoform diversity requirements through the analysis of conserved mechanosensory neuron afferent projections from adult fly bristles to the central nervous system (CNS). These neurons exhibit a highly elaborate and stereotypical branching pattern, providing a sensitive system for assessing Dscam isoform requirements in axon arbor formation (Figure 1). Using genetic mosaic techniques, *Dscam*-deficient mechanosensory axons are observed to reach the CNS but subsequently stall, with foreshortened axonal branches remaining clumped together near the CNS margin. Expression of single Dscam isoforms in identified, individual, mechanosensory neurons partially rescues this *Dscam* null phenotype, promoting axon extension further into the CNS. However, major defects remain, and the wild-type targeting pattern is not achieved. This suggests that Dscam plays an essential role in axon extension independent of isoform diversity, but accurate axon branching and targeting appear to have more complex Dscam requirements.

To what extent is the Dscam isoform diversity truly required for correct axonal branching and targeting? Does a significant reduction in the number of Dscam isoforms result in morphological defects in conserved axon branching patterns? To address this issue, two different *Dscam* deletion lines were generated, $Dscam^{\Delta R265}$ and $Dscam^{\Delta R272}$, that lack the exons 4.2–4.6 and 4.4–4.8, respectively (Figure 1). Despite the large number of isoforms still expressed in these deletion lines ($\sim$22,000 per line), individual identified mechanosensory neurons in both mutants exhibit pathfinding defects. These defects fall into various categories, including the presence of ectopic branches, misrouting of branches, and absence of certain branches normally observed in wild-type flies. Many of these defects are reproducible from animal to animal and are qualitatively distinct between these two deletion mutants (Figure 1). For example, $Dscam^{\Delta R265}$ mutants frequently show an ectopic branch in the prothoracic region of the post-Scutellar mechanosensory neuron rarely observed in $Dscam^{\Delta R272}$ mutants, whereas $Dscam^{\Delta R272}$ mutants exhibit an ectopic contralateral projection (Figure 1). These results are in contrast to previous findings demonstrating that partial deletion of the *Dscam* exon 4 cluster does not effect bifurcation of mushroom body neurons (Wang et al., 2004); however, this may simply result from distinct modes of Dscam isoform function in these two different experimental systems. In mushroom body neurons, Dscam present on branches of the same axon probably interacts in a homophilic manner, resulting in repulsive interactions. Any single Dscam isoform should be able to perform this function, though Dscam diversity, albeit of a nondeterministic nature, may be critical for correct mushroom body pathfinding in order to allow for self/nonself discrimination. In contrast, targeting of mechanosensory neurons may involve interactions among a specific set of Dscam isoforms present on mechanosensory axons and also along their trajectories or at their final targets. A prediction of this deterministic model is that, unlike in mushroom body neurons where it has been concluded that the expression of Dscam isoforms appears largely stochastic (Neves et al., 2004; Zhan et al., 2004), the expression profile of different Dscam isoforms in individual mechanosensory neurons may be unique, showing little animal-to-animal variation. Furthermore, a nonrandom pattern of Dscam isoform distribution might exist along mechanosensory neuron trajectories such that guidance information encoded by the Dscam isoform repertoire of each mechanosensory neuron can be effectively translated into a stereotypical axonal arbor patterning. Thus, only a defined set of Dscam isoforms may be capable of rescuing *Dscam* mutations in a given mechanosensory neuron, depending upon which Dscam isoforms are displayed along its trajectory. Indeed, such rescue studies represent an important direction for this current work on Dscam function in mechanosensory neurons. It has been proposed that strong homophilic interactions between Dscam isoforms lead to axonal repulsion, whereas weaker heterophilic interactions might mediate attraction (Wojtowicz et al., 2004). Therefore, mechanosensory neurons expressing defined sets of Dscam isoforms could be guided by repulsive interactions mediated by an identical set of Dscam isoforms expressed at crucial choice points along its trajec-

tory. Alternatively, mechanosensory neurons might be guided by adhesive interactions mediated by weak heterophilic interactions with Dscam isoforms present along their trajectories, with branching events controlled cell autonomously through repulsion mediated by homophilic interactions between Dscam isoforms. It will be crucial to determine which Dscam isoform functions required for generating disparate aspects of mechanosensory neuron morphology are repulsive or attractive, a goal which should be facilitated by molecular dissection of Dscam activity in this system.

The keen interest in Dscam arises from its extraordinary isoform diversity, and Chen et al. (2006) link Dscam isoform diversity to the generation of complex axonal trajectories. In addition to axon branching, Dscam is also critical for the morphogenesis of dendritic arbors (Zhu et al., 2006), raising the question of whether dendritic arbor complexity also requires Dscam isoform diversity. If indeed Dscam isoforms are expressed nonrandomly, it will also be extremely interesting to learn how precise regulation of *Dscam* alternative splicing occurs in a cell type-specific fashion. Understanding how Dscam regulates neuronal process morphology and how this may be coupled to restricted Dscam isoform expression will offer us valuable insight into how complex and distinct circuits are laid down during neural development.

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Circadian Transcription: Passing the HAT to CLOCK

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In animals, the circadian timekeeping mechanism relies on the coordinated activities of activators and repressors to control rhythmic transcription. In this issue of *Cell*, Doi et al. (2006) reveal that rhythms in histone acetylation are necessary for rhythmic transcription and that the histone acetyl transferase responsible is CLOCK, a key transcription factor that is essential for circadian oscillator function.

Circadian clocks control physiological, metabolic, and behavioral rhythms in organisms ranging from cyanobacteria to humans. Central to the function of these clocks is the circadian oscillator, which is set by environmental time cues but keeps circadian time in their absence. In eukaryotes, circadian oscillators generate rhythms via transcriptional feedback loops that mediate rhythmic transcription through the coordinated activity of transcriptional activators and repressors. In addition to controlling rhythmic expression of key feedback loop components, these feedback loops drive rhythmic expression of "output" genes that control physiological, metabolic, and behavioral rhythms.

In mammals and other vertebrates, heterodimers of the basic helix-loop-helix-PAS transcription factors CLOCK and BMAL1 activate transcription of the Period (*Per1* and *Per2*) and Cryptochrome (*Cry1* and *Cry2*) genes by binding E box sequences (reviewed in Lowrey and Takahashi [2004]). PER and CRY repressors then accumulate in